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Effect of Polybrene on Isolation of *Chlamydia trachomatis* from Clinical Specimens

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Polybrene treatment of McCoy cells was used to increase the infectivity of *Chlamydia trachomatis* in vitro. The isolation rate for *C. trachomatis* in 500 specimens from males and females attending a sexually transmitted disease clinic and other clinics increased by 6.8% with the Polybrene treatment. The data also suggested that this treatment facilitated the diagnosis of low grade or latent infections.

Genital infection due to *Chlamydia trachomatis* has become one of the most common sexually transmitted diseases (17). The organism is gram negative, has a unique growth cycle (1, 11), and requires complex techniques for its detection in clinical material. Most laboratories currently use tissue culture techniques for isolation, and their success varies with the quality, quantity, and method of collection of clinical material, temperature during transportation and storage of the specimen, laboratory media, and methodologies (10, 12).

Of the two species of the genus *Chlamydia*, *Chlamydia psittaci* and the three lymphogranuloma venereum serotypes (L1, L2, and L3) of *C. trachomatis* readily infect host cells in tissue culture, giving a high yield of progeny. The remaining 12 trachoma-inclusion conjunctivitis serotypes of *C. trachomatis* have been found to infect host cells poorly. Various physiochemical agents have been investigated during propagation in efforts to improve the isolation rate of *Chlamydia* spp. (5, 7, 8, 15, 19, 20).

Several investigators have reported enhancement of the size and yield of iodine-stained *C. trachomatis* inclusions when cycloheximide is incorporated into the cell culture medium (9, 14). Sabet et al. (16), studying the combined effect of DEAE-dextran and cycloheximide treatment on the number of *C. trachomatis* inclusions in HeLa 229 and McCoy cells by the indirect fluorescent antibody technique, observed an enhancement in HeLa 229 cells but a slight reduction in the number of inclusions in McCoy cells.

DEAE-dextran and cycloheximide enhancement of infectivity and yield of *C. trachomatis* in tissue culture varies with production lots and manufacturers (3, 13). Polybrene (1,5-dimethyl-1,5-diazaundecamethylene polymethobromide, hexadimethrine bromide; Sigma Chemical Co.), a relatively stable polycation, has been used to enhance retroviral infectivity in chick and mouse fibroblast cultures and appears to act by neutralization of the electrostatic charge on the cell surface, thus increasing adsorption and probable penetration of negatively charged microorganisms (18). The present study was undertaken to explore the effect of Polybrene on the infectivity of *C. trachomatis* in McCoy cells in efforts to improve the diagnosis of infections with this agent.

Initial experiments for observation of the effect of Polybrene on the cultivation of *C. trachomatis* in McCoy cells were done with five known tissue culture isolates from sexually transmitted disease (STD) patients after two passages in vitro and storage at −70°C. In the subsequent phase of the study, *C. trachomatis* isolation was attempted on 500 urethral and cervical swabs from patients attending a local STD clinic and other clinics run mostly by universities, gynecologists, obstetricians, and family physicians. The specimens were collected during November to December 1984 and transported to the laboratory on ice in sucrose-phosphate buffer medium within 24 h of collection.

The isolation procedure was carried out in microtiter plates (Nunc) as described by Yoder et al. (21) with cycloheximide at a final concentration of 1 μg/ml.

Polybrene at a final concentration of 4 μg/ml, based on results shown in Table 1, was added to Eagle minimum essential medium containing 2×10^5 McCoy cells per ml; 0.2 ml of this suspension was then transferred to each of the 96 wells of the microtiter plate. After 18 h of incubation at 37°C, the medium was removed and the wells were inoculated either in triplicate with 0.1 ml of laboratory-maintained, known isolates or in duplicate with clinical specimens.

On receipt, the clinical specimens were vortexed and divided into two aliquots. One was processed by the routine procedure of Yoder et al. (21) and observed for inclusions at ×200 under an Olympus inverted microscope after being stained with iodine. The other aliquot was inoculated in amounts of 0.05 and 0.1 ml into two wells, containing Polybrene-pretreated monolayers, and stained and read as above. Briefly, the inoculated plates were centrifuged at 1,100 × *g* for 1 h at 30°C and then incubated for another hour at 35°C. Specimen material was then aspirated and replaced with maintenance medium. The plates were left at 35°C for

TABLE 1. Effect of various concentrations of Polybrene on numbers of inclusions in McCoy cell monolayers with a laboratory strain of *C. trachomatis* (isolate 2)

Final concn of Polybrene (μg/ml)	No. of inclusions[a]	
	Pretreatment with Polybrene (18 h)	Polybrene and *C. trachomatis* added at same time
2	105.33 ± 15.00	32.00 ± 5.29
4	195.00 ± 7.00	12.00 ± 3.60
6	24.66 ± 6.10	5.33 ± 1.53
8	2.33 ± 2.08	0.66 ± 1.33
Control[b]		58.33 ± 8.5

[a] Each value indicates the mean of three wells ± standard deviation.
[b] No Polybrene added.

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TABLE 2. Comparison of number of inclusions of *C. trachomatis* in Polybrene-treated McCoy cell monolayers

C. trachomatis isolate no.	Mean no. of inclusions[a] in:		Factor of enhancement
	Untreated monolayer	Treated monolayer	
1	43.66 ± 5.03	121.66 ± 2.88	2.78
2	67.66 ± 2.51	179.33 ± 2.08	2.65
3	108.00 ± 12.52	225.33 ± 6.80	2.08
4	112.66 ± 9.01	333.66 ± 24.5	2.96
5	134.66 ± 9.23	244.00 ± 1.00	1.81

[a] Each value indicates the mean of three wells ± standard deviation.

48 h and stained with a 10% solution of Lugol's iodine in methanol. The two aliquots were processed independently in a blind study. Statistical analyses were carried out by the chi-square test with Yates correction.

Pretreatment of McCoy cells with Polybrene enhanced the number of inclusions by approximately 2.5 times, as compared with untreated cells (Table 2). The enhancement was consistent with all five laboratory isolates of *C. trachomatis*. The size of the inclusions in untreated and treated McCoy cells did not vary significantly.

The isolation of *C. trachomatis* from clinical specimens was studied in Polybrene-treated and untreated McCoy cells. Of 500 specimens, 88 (17.6%) and 54 (10.8%) were positive for *C. trachomatis* in treated and untreated cells, respectively. Thirty-four (6.8%) positive specimens were detected only in the Polybrene-treated McCoy cells. All specimens positive in untreated cells were also positive in Polybrene-treated cells.

Table 3 shows the frequency of isolation of *C. trachomatis* by type of clinic. It is interesting that the effect of Polybrene on the isolation rate of *C. trachomatis* in specimens from other clinics was more significant than the one from the STD clinic.

As the quality of specimen varies with age and sex of the patient, we decided to analyze data based on these parameters. In general, the pattern and isolation rate of *C. trachomatis* in females aged 15 to 30 years attending the STD clinic were not affected after Polybrene pretreatment (Table 4). However, significant differences were noted among the males as a group ($P < 0.05$): 45 of 271 (16.6%) specimens were positive with Polybrene-treated cells compared with 25 of 271 (9.2%) positive specimens for the untreated cells. The same was true for males between the ages of 21 and 30, in whom the isolation rate was seen to increase by almost 100%.

In the group of females attending other clinics, the isolation rate in both Polybrene-pretreated and untreated McCoy cells was low when compared with that of the STD group. The increase in the isolation rate due to Polybrene treatment was statistically significant in the 21-to-25-year age group ($P < 0.05$).

TABLE 3. Isolation of *C. trachomatis* with Polybrene-treated McCoy cells: distribution by clinic

Clinic	Total no. of specimens	Specimens positive for *C. trachomatis* in[a]:		Factor of improvement
		Untreated monolayers	Treated monolayers	
STD	386	48 (12.4)	72 (18.6)	1.5
Other	114	6 (5.2)	16 (14.0)	2.7

[a] Values in parentheses indicate percentage of total.

TABLE 4. Frequency of isolation of *C. trachomatis* by age and sex of patients attending the STD clinic and other clinics

Sex and age group of patients (*n*)	Positive cultures in[a]:	
	Untreated monolayers	Treated monolayers
Males[b]		
15–20 yr (26)	7 (27)	8 (30.7)
21–25 yr (93)	9 (9.7)	18 (19.4)
26–30 yr (57)	7 (12.3)	12 (21.1)
31–40 yr (65)	1 (1.5)	4 (6.1)
40+ yr (30)	1 (3.3)	3 (10)
Females		
STD clinic		
15–20 yr (33)	9 (27.3)	10 (30.3)
21–25 yr (47)	10 (21.3)	11 (23.4)
26–30 yr (18)	4 (22.2)	5 (27.8)
31–40 yr (15)	0	1 (6.6)
40+ yr (2)	0	0
Other clinics		
15–20 yr (11)	1 (9.1)	2 (18.2)
21–25 yr (24)	3 (12.5)	7 (29.2)
26–30 yr (27)	2 (7.4)	3 (11.1)
31–40 yr (30)	0	2 (6.7)
40+ yr (11)	0	0
Total %		
Males	9.2	16.6
Females		
STD clinic	20.9	23.47
Other clinics	5.8	13.6

[a] Values in parentheses indicate percentage of total specimens.
[b] Eleven males not included (insignificant number).

The effective propagation of *C. trachomatis* in tissue culture and its isolation from clinical specimens are influenced by various factors, some of which are still ill-defined. Probably less than 1% of all the infective particles in clinical specimens produce detectable inclusions (6), even though natural infection in humans is readily transmissible by brief contact. Due to an incomplete understanding of the biology of *Chlamydia*, a universal standardized technique is not yet available, which results in inconsistent isolation rates from various laboratories with different tissue culture cell lines grown in different media under different conditions of treatment.

The attachment of *C. trachomatis* to the host cell is important for its adsorption, phagocytosis, and penetration. Treatments such as centrifugation (4, 19) and neutralization of electrostatic charges between *C. trachomatis* and host cells by polycations (7) have been used to enhance attachment. Sabet et al. (16), working with *C. trachomatis* serotype G, observed an increase in the number of infected HeLa 229 cells pretreated with DEAE-dextran. They also found an increase in both the number of infected cells and *C. trachomatis* progeny yield when this pretreatment was combined with an additional treatment with cycloheximide. Our data with tissue-culture-grown *C. trachomatis* strains (Table 2) and clinical specimens (Table 3) suggest that Polybrene probably acts in a way similar to that of DEAE-dextran by reducing repulsive electronegative charges between *C. trachomatis* and the host cell, thus assisting in the adsorption of the agent onto the cell. However, in contrast to the observations of Sabet et al. with McCoy cells treated with DEAE-dextran alone or combined with cycloheximide (16),

we found an enhancement in the number of inclusions after pretreatment with Polybrene at a concentration of 4 μg/ml. Polybrene at a higher concentration of 8 μg/ml showed an inhibitory effect with little host cell infectivity by *C. trachomatis* (Table 1). Adding Polybrene simultaneously with *C. trachomatis* also had an inhibitory effect on the infectious process (Table 1).

Polybrene pretreatment increased the detection of positive specimens in two groups, females attending other clinics and males attending the STD clinic. In women, the presence of discharge, mucus, bacterial products, enzymes, toxic substances, and locally produced antibodies could contribute to low virulence of *C. trachomatis* or a latent status or both. In men, owing to the simple structure of the urethra, acute symptoms may manifest themselves even in the presence of relatively low numbers of *Chlamydia* particles, prompting them to seek medical attention at an early stage of infection. The higher isolation rate of *C. trachomatis* in females attending the STD clinic may be due to increased frequency and dose of chlamydial inoculation as a consequence of their lifestyles and behavioral patterns.

Twenty males and four females from the STD clinic and two males and eight females from other clinics that were positive only on the Polybrene-treated McCoy cells had low inclusion counts; from this total of 34 patients, 24 had inclusion counts between one to three per monolayer, 8 had counts between four to eight inclusions, and the remaining 2 had eight and ten inclusions per monolayer, respectively. These *C. trachomatis* organisms in low numbers were probably not adsorbed onto the untreated McCoy cells under routine conditions and remained undetected.

Although further studies are needed, we feel that, due to its stable character, nontoxic effect, and low cost, Polybrene can be used to improve isolation of *C. trachomatis*, especially from specimens in which the organism is present in low numbers.

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